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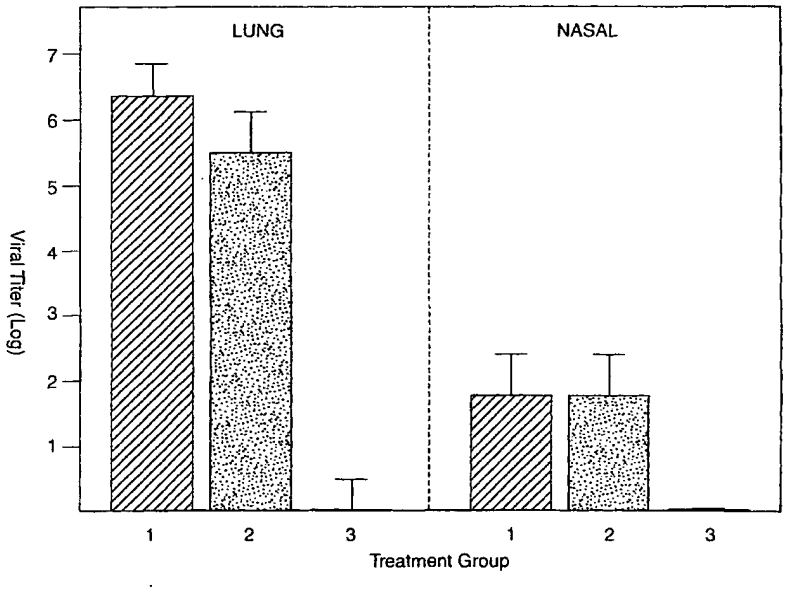
(54) Title: INFECTION PROPHYLAXIS USING IMMUNE RESPONSE MODIFIER COMPOUNDS



(57) Abstract: The present invention provides methods of providing prophylaxis to a subject against an infectious agent. In general, the methods include topically administering to the respiratory tract of a subject an IRM compound in an amount effective to reduce infection by the agent.

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INFECTION PROPHYLAXIS USING IMMUNE RESPONSE MODIFIER COMPOUNDS

Background

Immune response modifiers ("IRMs") include compounds that possess potent immunomodulating activity including but not limited to antiviral and antitumor activity. Certain IRMs affect immune activity by modulating the production and secretion of cytokines. For example, cytokines that are induced by certain small molecule IRM compounds include but are not limited to Type I interferons, TNF-α, IL-1, IL-6, IL-8, IL-10, IL-12, MIP-1, and MCP-1. Alternatively, certain IRM compounds can inhibit production and secretion of certain T_H2 cytokines such as IL-4 and IL-5.

By stimulating certain aspects of the immune system, suppressing other aspects, or both, IRMs may be used to treat many diseases and conditions. For example, IRM compounds may be useful for treating viral diseases, neoplasias, fungal diseases, neoplastic diseases, parasitic diseases, atopic diseases, and opportunistic infections and tumors that occur after suppression of cell-mediated immunity. IRM compounds also may be useful for promoting healing of wounds and post-surgical scars. Specifically, but not exclusively, diseases that may be treated using IRM compounds include, but are not limited to, external genital and perianal warts caused by human papillomavirus, basal cell carcinoma, eczema, essential thrombocythaemia, hepatitis B, multiple sclerosis, neoplastic diseases, atopic dermatitis, asthma, allergies, psoriasis, rheumatoid arthritis, type I herpes simplex, and type II herpes simplex.

Certain formulations of a small molecule imidazoquinoline IRM compound have been shown to be useful for the therapeutic treatment of certain cancerous or pre-cancerous lesions (See, e.g., Geisse *et al.*, *J. Am. Acad. Dermatol.*, 47(3): 390-398 (2002); Shumack *et al.*, *Arch. Dermatol.*, 138: 1163-1171 (2002); U.S. Pat. No. 5,238,944; and WO 03/045391).

IRM compounds also can modulate humoral immunity by stimulating antibody production by B cells. Further, various IRMs have been shown to be useful as vaccine adjuvants (see, e.g., U.S. Pat. Nos. 6,083,505 and 6,406,705).

Certain IRM compounds are known to be agonists of at least one Toll-like receptor (TLR). For example, IRM compounds are known to be an agonist of TLR6, TLR7, TLR8,

or some combination thereof. Also, for example, certain modified oligonucleotide IRM compounds are known to be agonists of TLR9.

Summary

It has been found that certain IRMs can be used to provide prophylaxis against an infectious agent when topically administered to the respiratory tract of a subject. Moreover, infection prophylaxis may be provided regardless of whether the IRM is administered before or after the subject is exposed to the infectious agent.

Accordingly, the present invention provides a method of providing prophylaxis to a subject against an infectious agent. The method includes topically administering to the respiratory tract of a subject an IRM compound in an amount effective to limit infection by the agent. In some embodiments, the IRM compound can be administered from about 72 hours prior to exposure to the infectious agent to about 72 hours after exposure to the infectious agent.

Various other features and advantages of the present invention should become readily apparent with reference to the following detailed description, examples, claims and appended drawings. In several places throughout the specification, guidance is provided through lists of examples. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

Brief Description of the Drawings

Fig. 1 is a bar graph comparing viral titers in rats after treatment with vehicle, IFN-α, or IRM compound four hours before viral challenge.

Fig. 2 is a bar graph comparing viral titers in rats after treatment with vehicle, IFN-α, or IRM compound twenty-four hours and again at four hours before viral challenge.

Detailed Description of Illustrative Embodiments of the Invention

The present invention relates to methods of providing prophylaxis against an infectious agent by topically administering an IRM compound to the respiratory system of a subject. In some embodiments of the present invention, the IRM compound is administered in a relatively limited dosing regimen that is specifically initiated upon actual, suspected, or anticipated possible exposure to an infectious agent. Therefore, certain methods of the present invention may be particularly suited for providing infection

prophylaxis for those who have been, intend to be, or suspect that they may be exposed to an infectious agent.

For purposes of this invention, the following terms shall have the meanings set forth as follows:

"Agonist" refers to a compound that can combine with a receptor (e.g., a TLR) to induce a cellular activity. An agonist may be a ligand that directly binds to the receptor. Alternatively, an agonist may combine with a receptor indirectly by, for example, (a) forming a complex with another molecule that directly binds to the receptor, or (b) otherwise results in the modification of another compound so that the other compound directly binds to the receptor. An agonist may be referred to as an agonist of a particular TLR (e.g., a TLR6 agonist) or a particular combination of TLRs (e.g., a TLR 7/8 agonist – an agonist of both TLR7 and TLR8).

"Cellular activity" refers to a biological activity (e.g., cytokine production), the modulation of which results from an agonist-receptor interaction or an antagonist-receptor interaction.

"Exposure" of a subject to a pathogen refers to actual or anticipated contact between the subject and the pathogen. "Actual exposure" refers to exposure in fact, whether known or unknown. "Anticipated exposure" refers to any level of expected possibility of being exposed to a pathogen.

"Induce" and variations thereof refer to any measurable increase in cellular activity. Thus, as used herein, "induce" or "induction" may be referred to as a percentage of a normal level of activity. In the case of a cell product such as, for example, a cytokine, chemokine, or co-stimulatory marker, "induce" also may refer to any measurable increase in the amount of the cell product that is produced in response to a stimulus.

"Prophylaxis" refers to any degree of limiting an infection by an infectious agent including (a) preventing or limiting an initial infection, (b) preventing or limiting the spread of an existing infection, or both. "Prophylaxis" may be used interchangeably with "reduce infection" and variations thereof.

"Respiratory Tract" refers, generally, to the major structures and passages that permit or provide air flow between the environment and the lungs of a subject. "Upper Respiratory Tract" refers, generally, to the nasal cavity, paranasal sinuses, nasopharynx,

oral cavity, pharynx, and larynx. "Lower Respiratory Tract" refers, generally, to the trachea and lungs, including the bronchi, bronchioles, and alveoli.

"TLR-mediated" refers to a biological or biochemical activity that results, directly or indirectly, from TLR function. A particular biological or biochemical activity may be referred to as mediated by a particular TLR (e.g., "TLR6-mediated" or "TLR7-mediated").

"Topical" refers to administering the IRM compound to a surface of the respiratory tract. Topical administration to the respiratory tract can occur via formulations including but not limited to an aerosol, a non-aerosol spray, a cream, an ointment, a gel, a lotion, a mouthwash, and the like.

The methods of the present invention can provide general prophylaxis against infection by an infectious agent. One feature of certain methods of the present invention is that topical administration of the IRM compound can provide infection prophylaxis against one or a plurality of known or unknown infectious agents. Knowledge or even suspicion of the identity of the particular infectious agent to which one has or might be exposed is not required. Accordingly, the methods of the present invention may be particularly useful when exposure to an infectious agent has occurred, is suspected to have occurred, or is anticipated, but the identity of the particular infectious agent is not known. Events in which the methods of the present invention thus may be particularly useful include, but are not limited to, outbreaks of new or previously unidentified infectious agents, biological warfare, bio-terrorism, and exposure to environments that can have a relatively large number of different infectious agents (e.g., health care clinics, daycare centers, and the like).

In certain embodiments, it may be possible to enhance infection prophylaxis against a particular infectious agent, if known, by combining administration of the IRM compound with separate or co-administration of one or more components of the infectious agent. However, infection prophylaxis using the methods of the present invention does not require such combinations or – as stated above - even knowledge of the identity of the infectious agent. Another feature of certain embodiments of the present invention is the ease and relatively limited discomfort associated with administration of the IRM compound. Topical administration to the respiratory tract can involve inhalation of an aerosol or non-aerosol formulation, the inhaled formulation being deposited on the surface of structures of the upper respiratory tract, the lower respiratory tract, or both.

Alternatively, topical administration of the IRM compound to the respiratory tract may involve contacting a surface of the respiratory tract – typically, the upper respiratory tract – with a cream, gel, mouthwash, or the like. The formulation may be left in place following administration (e.g., a cream or gel applied to a surface of the oral or nasal cavity) or may be discarded (e.g., a mouthwash). Administration of the IRM compound can be minimally invasive, particularly when compared to subcutaneous, intramuscular, or transdermal vaccination.

Yet another feature of certain methods of the present invention is that administration of the IRM compound can be temporally connected to an exposure event, whether the exposure event has occurred, is suspected of having occurred, or is expected to occur. Moreover, infection prophylaxis can be provided without requiring administration of a prophylactic agent over weeks or months of treatment. For example, in some embodiments, infection prophylaxis can be provided by one or two doses of IRM compound administered about 72 hours or less before an anticipated exposure to an infectious agent. In some embodiments, infection prophylaxis can be provided by a single dose of IRM compound administered four hours before exposure to the infectious agent.

Alternatively, infection prophylaxis can be provided by administering the IRM compound after exposure to the infectious agent has occurred or is suspected to have occurred. For example, in some embodiments, infection prophylaxis can be provided by one or two doses of IRM compound administered about 72 hours or less after an actual or suspected exposure event. In certain embodiments, infection prophylaxis can be provided with a single dose of IRM compound provided within 24 hours of the exposure event. In

many embodiments, infection prophylaxis can be provided without requiring administration of a prophylactic or therapeutic agent over weeks or months of treatment.

In certain embodiments, administration of IRM compound before an anticipated exposure event may be combined with administration of IRM compound after an actual or suspected exposure event.

The features of certain embodiments of the present invention can be particularly desirable for some applications. For example, each of: (a) the temporal connection to an exposure event, (b) ease of administration, and (c) relatively short dosing regimens – alone and in various combinations – can increase the likelihood and extent of compliance, thereby increasing the efficacy of the prophylaxis.

As another example, the general infection prophylaxis conferred by administering IRM compound renders certain embodiments useful for providing infection prophylaxis even when the number and identity of infectious agent(s) is unknown. Thus, methods of the present invention may be particularly desirable in circumstances when, at the time when a standard vaccination would have to be given to provide infection prophylaxis, it is unclear to which, if any, infectious agents one may be exposed. Thus, infection prophylaxis using methods of the present invention can be provided on an "as needed" basis, thereby reducing the number of vaccinations administered unnecessarily.

Additionally, a single course of administering IRM compound may provide at least as great a scope - and in some cases, even greater scope - of infection prophylaxis as several vaccinations, with reduced costs (e.g., less developmental cost, one administration versus several, etc.), less discomfort, and without the inherent risk associated with standard vaccinations. All of the above factors may contribute to increased compliance compared to traditional vaccinations, which, in turn, may result in a higher percentage of individuals in a treated population having efficacious infection prophylaxis.

Infectious agents against which IRM compounds may provide infection prophylaxis using methods of the present invention include, but are not limited to: (a) viruses such as, for example, variola, HIV, CMV, VZV, rhinovirus, adenovirus, coronavirus (including, e.g., SARS), influenza, para-influenza, ebola, hepatitis B, hepatitis C, and West Nile virus; (b) bacteria, such as those that can cause tuberculosis, anthrax, listeriosis, and leprosy; (c) other infectious agents such as prions and parasites (e.g., *Leishmania* spp.);

For example, certain methods of the present invention may be particularly useful for daycare or health care workers who have been or will be exposed to an infectious agent. Thus, certain methods of the present invention may secondarily reduce sick time taken by employees in certain industries.

As another example, certain methods of the present invention may be particularly useful for providing prophylaxis against biological warfare or bio-terrorism agents. Such methods may be employed by military personnel either (a) before an anticipated exposure to a biological warfare agent, (b) before entering an area where a biological warfare agent has previously been used, or (c) after a suspected or known exposure to a biological warfare agent. Certain methods of the present invention may be particularly useful in the event of exposure to a bio-terrorism agent. In some cases, the methods may be employed by emergency personnel including police, medical personnel, firefighters, and the like. In other cases, the methods may be employed by the general population or a subset of the general population. In either case, certain methods may provide infection prophylaxis when employed during or after either (a) exposure to a bio-terrorism agent, or (b) a warning that a bio-terrorism event may occur.

As yet another example, the invention may be practiced to provide infection prophylaxis for those who must – or choose to – travel to an area experiencing an outbreak of an infectious agent. For example, health care workers may travel to an area experiencing such an outbreak to care for those affected by the infectious agent. Others may choose not to cancel or delay business or pleasure travel plans. In these cases, practicing the invention may help provide infection prophylaxis to those traveling into the area of an outbreak, thereby, for example, facilitating health care for those affected, minimizing the economic and social cost of the outbreak, or both.

Type 1 interferons such as, for example, IFN-α have been shown to possess antiviral activity, perhaps by induction of a nonspecific antiviral state. Administration of IFN-α can be effective, as a monotherapy, preventing some viral infections. However, IFN-α monotherapy has not gained widespread acceptance, perhaps at least in part due to associated side-effects such as, for example, nasal stuffiness, increased sneezing, bleeding, and nasal erosion.

Figures 1 and 2 summarize data demonstrating the efficacy of the invention in providing infection prophylaxis against viral infection. Administration of the IRM

compound prior to intranasal infection with influenza virus dramatically reduced the viral titer present in the lung and nasal cavity 24 hours after the infection, even compared to pretreatment with IFN-α.

Table 3 summarizes data that demonstrates the efficacy of IRM compound preventing replication of various viruses. IRM compound was used to inhibit viral replication in each of two ways: directly and indirectly. Indirect inhibition of viral replication occurred when IRM compound was used to stimulate a culture of peripheral blood mononuclear cells (PBMCs). Factors secreted by the PBMCs into the culture supernatant were collected and tested for the ability to inhibit viral replication.

Inhibition of viral replication occurred with PBMC supernatant solutions at approximate EC_{50} values of 0.17 μM against coronavirus, <0.0015 μM against influenza A/Panama/2007/99, <0.0015 μM against influenza A/New Caledonia/20/99, and 0.15 μM against SARS.

Direct inhibition of viral replication occurred when IRM compound itself was tested for the ability to inhibit viral replication. IRM compound alone added to media was able to inhibit SARS replication with an approximate EC_{50} at 3 μM.

The results of these studies indicate that IRM compounds stimulate significant antiviral factors to be secreted from PBMCs into supernatant solutions. When considered together with the data from Figs. 1 and 2, the antiviral properties associated with practicing the invention are greater than the antiviral properties of IFN-α monotherapy. These data support the use of IRM compounds for providing non-specific prophylactic and/or therapeutic treatment of viral infections.

Immune response modifiers ("IRMs") include compounds that possess potent immunomodulating activity including but not limited to antiviral and antitumor activity. Certain IRMs modulate the production and secretion of cytokines. For example, certain IRM compounds induce the production and secretion of cytokines such as, e.g., Type I interferons, TNF-α, IL-1, IL-6, IL-8, IL-10, IL-12, MIP-1, and/or MCP-1. As another example, certain IRM compounds can inhibit production and secretion of certain T_H2 cytokines, such as IL-4 and IL-5. Additionally, some IRM compounds are said to suppress IL-1 and TNF (U.S. Patent No. 6,518,265).

Certain IRMs are small organic molecules (e.g., molecular weight under about 1000 Daltons, preferably under about 500 Daltons, as opposed to large biological

molecules such as proteins, peptides, and the like) such as those disclosed in, for example, U.S. Patent Nos. 4,689,338; 4,929,624; 4,988,815; 5,037,986; 5,175,296; 5,238,944; 5,266,575; 5,268,376; 5,346,905; 5,352,784; 5,367,076; 5,389,640; 5,395,937; 5,446,153; 5,482,936; 5,693,811; 5,741,908; 5,756,747; 5,939,090; 6,039,969; 6,083,505; 6,110,929; 6,194,425; 6,245,776; 6,331,539; 6,376,669; 6,451,810; 6,525,064; 6,541,485; 6,545,016; 6,545,017; 6,558,951; 6,573,273; 6,656,938; 6,660,735; 6,660,747; 6,664,260; 6,664,264; 6,664,265; 6,667,312; 6,670,372; 6,677,347; 6,677,348; 6,677,349; 6,683,088; 6,756,382; European Patent 0 394 026; U.S. Patent Publication Nos. 2002/0016332; 2002/0055517; 2002/0110840; 2003/0133913; 2003/0199538; and 2004/0014779; and International Patent Publication Nos. WO 01/74343; WO 02/46749 WO 02/102377; WO 03/020889; WO 03/043572; WO 03/045391; WO 03/103584; and WO 04/058759.

Additional examples of small molecule IRMs include certain purine derivatives (such as those described in U.S. Patent Nos. 6,376,501, and 6,028,076), certain imidazoquinoline amide derivatives (such as those described in U.S. Patent No. 6,069,149), certain imidazopyridine derivatives (such as those described in U.S. Patent No. 6,518,265), certain benzimidazole derivatives (such as those described in U.S. Patent 6,387,938), certain derivatives of a 4-aminopyrimidine fused to a five membered nitrogen containing heterocyclic ring (such as adenine derivatives described in U. S. Patent Nos. 6,376,501; 6,028,076 and 6,329,381; and in WO 02/08905), and certain 3-β-D-ribofuranosylthiazolo[4,5-d]pyrimidine derivatives (such as those described in U.S. Publication No. 2003/0199461).

Other IRMs include large biological molecules such as oligonucleotide sequences. Some IRM oligonucleotide sequences contain cytosine-guanine dinucleotides (CpG) and are described, for example, in U.S. Patent Nos. 6,194,388; 6,207,646; 6,239,116; 6,339,068; and 6,406,705. Some CpG-containing oligonucleotides can include synthetic immunomodulatory structural motifs such as those described, for example, in U.S. Patent Nos. 6,426,334 and 6,476,000. Other IRM nucleotide sequences lack CpG sequences and are described, for example, in International Patent Publication No. WO 00/75304.

Other IRMs include biological molecules such as aminoalkyl glucosaminide phosphates (AGPs) and are described, for example, in U.S. Patent Nos. 6,113,918; 6,303,347; 6,525,028; and 6,649,172.

The IRM compound may be any suitable IRM compound. In some embodiments of the present invention, the IRM compound may include a 2-aminopyridine fused to a five membered nitrogen-containing heterocyclic ring, or a 4-aminopyrimidine fused to a five membered nitrogen-containing heterocyclic ring. In some embodiments, suitable IRM compounds include but are not limited to the small molecule IRM compounds (e.g., molecular weight of less than about 1000 Daltons) described above.

Certain small molecule IRM compounds – those having a 2-aminopyridine fused to a five membered nitrogen-containing heterocyclic ring – include, for example, imidazoquinoline amines including but not limited to substituted imidazoquinoline amines; tetrahydroimidazoquinoline amines including but not limited to amide substituted tetrahydroimidazoquinoline amines, sulfonamide substituted tetrahydroimidazoquinoline amines, urea substituted tetrahydroimidazoquinoline amines, aryl ether substituted tetrahydroimidazoquinoline amines, heterocyclic ether substituted tetrahydroimidazoquinoline amines, amido ether substituted tetrahydroimidazoquinoline amines, sulfonamido ether substituted tetrahydroimidazoquinoline amines, urea substituted tetrahydroimidazoquinoline ethers, and thioether substituted tetrahydroimidazoquinoline amines; imidazopyridine amines including but not limited to amide substituted imidazopyridine amines, sulfonamide substituted imidazopyridine amines, urea substituted imidazopyridine amines, aryl ether substituted imidazopyridine amines, heterocyclic ether substituted imidazopyridine amines, amido ether substituted imidazopyridine amines, sulfonamido ether substituted imidazopyridine amines, urea substituted imidazopyridine ethers, and thioether substituted imidazopyridine amines; 1,2-bridged imidazoquinoline amines; 6,7-fused cycloalkylimidazopyridine amines; imidazonaphthyridine amines; tetrahydroimidazonaphthyridine amines; oxazoloquinoline amines; thiazoloquinoline amines; oxazolopyridine amines; thiazolopyridine amines; oxazolonaphthyridine amines; thiazolonaphthyridine amines; and 1*H*-imidazo dimers fused to pyridine amines, quinoline amines, tetrahydroquinoline amines, naphthyridine amines, or tetrahydronaphthyridine amines.

In certain embodiments, the IRM compound may be an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, or a thiazolonaphthyridine amine.

In certain embodiments, the IRM compound may be a substituted imidazoquinoline amine, a tetrahydroimidazoquinoline amine, an imidazopyridine amine, a 1,2-bridged imidazoquinoline amine, a 6,7-fused cycloalkylimidazopyridine amine, an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, or a thiazolonaphthyridine amine.

As used herein, a substituted imidazoquinoline amine refers to an amide substituted imidazoquinoline amine, a sulfonamide substituted imidazoquinoline amine, a urea substituted imidazoquinoline amine, an aryl ether substituted imidazoquinoline amine, a heterocyclic ether substituted imidazoquinoline amine, an amido ether substituted imidazoquinoline amine, a sulfonamido ether substituted imidazoquinoline amine, a urea substituted imidazoquinoline ether, a thioether substituted imidazoquinoline amines, or a 6-, 7-, 8-, or 9-aryl or heteroaryl substituted imidazoquinoline amine. As used herein, substituted imidazoquinoline amines specifically and expressly exclude 1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine and 4-amino-α,α-dimethyl-2-ethoxymethyl-1*H*-imidazo[4,5-*c*]quinolin-1-ethanol.

In certain embodiments, the IRM compound can be a sulfonamide substituted imidazoquinoline amine. In certain specific embodiments, the IRM compound can be N-{2-[4-amino-2-(ethoxymethyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl]-1,1-dimethylethyl}methanesulfonamide.

Suitable IRM compounds also may include the purine derivatives, imidazoquinoline amide derivatives, benzimidazole derivatives, adenine derivatives, and oligonucleotide sequences described above.

In some embodiments, the IRM compound may be a compound identified as an agonist of one or more TLRs. In some embodiments of the present invention, the IRM compound may be an agonist of at least one TLR, preferably an agonist of TLR6, TLR7, or TLR8. The IRM compound may, in some cases, be an agonist of TLR 9. In certain embodiments, the IRM compound may be an agonist of at least TLR7 – i.e., may be, for example, a TLR7/8 agonist or a TLR7-selective agonist.

As used herein, the term "TLR7/8 agonist" refers to a compound that acts as an agonist of both TLR7 and TLR8. A "TLR7-selective agonist" refers to a compound that acts as an agonist of TLR7, but does not act as an agonist of TLR8. Thus, a "TLR7-

selective agonist" may refer to a compound that acts as an agonist for TLR7 and for no other TLR, but it may alternatively refer to a compound that acts as an agonist of TLR7 and a TLR other than TLR8 such as, for example, TLR6.

The TLR agonism for a particular compound may be assessed in any suitable manner. For example, assays for detecting TLR agonism of test compounds are described, for example, in U.S. Patent Application Ser. No. 10/732,563, filed December 10, 2003, and recombinant cell lines suitable for use in such assays are described, for example, in U.S. Patent Application Ser. No. 10/732,796, filed December 10, 2003.

Regardless of the particular assay employed, a compound can be identified as an agonist of a particular TLR if performing the assay with a compound results in at least a threshold increase of some biological activity mediated by the particular TLR. Conversely, a compound may be identified as not acting as an agonist of a specified TLR if, when used to perform an assay designed to detect biological activity mediated by the specified TLR, the compound fails to elicit a threshold increase in the biological activity. Unless otherwise indicated, an increase in biological activity refers to an increase in the same biological activity over that observed in an appropriate control. An assay may or may not be performed in conjunction with the appropriate control. With experience, one skilled in the art may develop sufficient familiarity with a particular assay (e.g., the range of values observed in an appropriate control under specific assay conditions) that performing a control may not always be necessary to determine the TLR agonism of a compound in a particular assay.

The precise threshold increase of TLR-mediated biological activity for determining whether a particular compound is or is not an agonist of a particular TLR in a given assay may vary according to factors known in the art including but not limited to the biological activity observed as the endpoint of the assay, the method used to measure or detect the endpoint of the assay, the signal-to-noise ratio of the assay, the precision of the assay, and whether the same assay is being used to determine the agonism of a compound for both TLRs. Accordingly it is not practical to set forth generally the threshold increase of TLR-mediated biological activity required to identify a compound as being an agonist or a non-agonist of a particular TLR for all possible assays. Those of ordinary skill in the art, however, can readily determine the appropriate threshold with due consideration of such factors.

Assays employing HEK293 cells transfected with an expressible TLR structural gene may use a threshold of, for example, at least a three-fold increase in a TLR-mediated biological activity (e.g., NFκB activation) when the compound is provided at a concentration of, for example, from about 1 μM to about 10 μM for identifying a compound as an agonist of the TLR transfected into the cell. However, different thresholds and/or different concentration ranges may be suitable in certain circumstances. Also, different thresholds may be appropriate for different assays.

The IRM compound may be provided in a formulation suitable for topical administration to the respiratory tract of a subject. The IRM compound may be provided in any suitable form including but not limited to a solution, a suspension, an emulsion, a dry powder, or any form of mixture. The IRM compound may be administered in formulation with any pharmaceutically acceptable excipient, carrier, or vehicle. The formulation may be administered in any conventional dosage form for topical delivery to a surface of the respiratory tract. Such dosage forms include but are not limited to an aerosol formulation, a non-aerosol spray, a cream, an ointment, a gel, a lotion, a mouthwash, and the like. Suitable aerosol formulations are described, for example, in U.S. Pat. No. 6,126,919. Alternative formulations are described, for example, in U.S. Pat. No. 5,238,944; EP 0 394 026; U.S. Pat. No. 6,365,166; and U.S. Pat. No. 6,245,776. The formulation may further include one or more additives including but not limited to adjuvants, penetration enhancers, colorants, fragrances, moisturizers, thickeners, and the like.

In some embodiments, the methods of the present invention include administering IRM compound to a subject in a formulation of, for example, from about 0.001% to about typically 10% (unless otherwise indicated, all percentages provided herein are weight/weight with respect to the total formulation) to the subject, although in some embodiments the IRM compound may be administered using a formulation that provides IRM compound in a concentration outside of this range. In certain embodiments, the method includes administering to a subject a formulation that includes from about 0.01% to about 1.0 % IRM compound, for example, a formulation that includes about 0.375% IRM compound.

An amount of an IRM compound effective for providing infection prophylaxis is an amount sufficient to either prevent or limit (a) an initial infection, or (b) the spread of

an existing infection, or both. One method of determining an amount effective for providing infection prophylaxis is to determine an amount effective to reduce – to a statistically significant extent – nasal or lung titers of the infectious agent 24 hours after the latter of: (a) exposure to the infectious agent, or (b) administration of the IRM compound.

The precise amount of IRM compound in a dose may vary according to factors known in the art including but not limited to the physical and chemical nature of the IRM compound, the nature of the carrier, the intended dosing regimen, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method of administering the IRM compound, the species to which the formulation is being administered, and the infectious agent or agents, if known, to which the subject has been or is expected to be exposed. Accordingly it is not practical to set forth generally the amount that constitutes an amount of IRM compound effective for providing infection prophylaxis for all possible applications. Those of ordinary skill in the art, however, can readily determine the appropriate amount with due consideration of such factors.

In some embodiments, the methods of the present invention include administering sufficient IRM compound to provide a dose of, for example, from about 100 ng/kg to about 50 mg/kg to the subject, although in some embodiments the methods may be performed by administering IRM compound in concentrations outside this range. In some of these embodiments, the method includes administering sufficient IRM compound to provide a dose of from about 10 μg/kg to about 5 mg/kg to the subject, for example, a dose of from about 100 μg/kg to about 1 mg/kg.

The dosing regimen may depend at least in part on many factors known in the art including but not limited to the physical and chemical nature of the IRM compound, the nature of the carrier, the amount of IRM compound being administered, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method of administering the IRM compound, the species to which the formulation is being administered, and the infectious agent or agents, if known, to which the subject has been or is expected to be exposed. Accordingly it is not practical to set forth generally the dosing regimen effective to provide infection prophylaxis for all possible applications. Those of ordinary skill in the art, however, can readily determine the appropriate amount with due consideration of such factors.

In some embodiments of the invention, the IRM compound may be administered, for example, from once to about twelve times over the entire course of treatment, although in some embodiments the methods of the present invention may be performed by administering the IRM compound at a frequency outside this range. In certain embodiments, the IRM compound may be administered from once to about four times over the entire course of treatment. In one particular embodiment, the IRM compound is administered once. In another particular embodiment, the IRM compound is administered twice.

The IRM compound may be administered before an anticipated exposure to the infectious agent. In some embodiments, the IRM compound is administered once per day for a period before an anticipated exposure to the infectious agent. In certain embodiments, the IRM compound may be administered once per day for two days. In other embodiments, the IRM compound may be administered in a single dose. In some embodiments, at least one dose of the IRM compound is administered 72 hours or less before an anticipated exposure to the infectious agent. In one particular embodiment, for example, the IRM compound is administered in a single dose, about 4 hours prior to exposure to the infectious agent.

In alternative embodiments, the IRM compound may be administered after a suspected or confirmed exposure to the infectious agent. In these embodiments, the IRM compound may be administered once per day for a period after a suspected or actual exposure to the infectious agent. In certain embodiments, the IRM compound may be administered once per day for two days. In other embodiments, the IRM compound may be administered in a single dose. In some embodiments, at least one dose of the IRM compound is administered 72 hours or less after the actual or suspected exposure to the infectious agent. In one particular embodiment, for example, the IRM compound is administered in a single dose, about 4 hours after exposure to the infectious agent.

In embodiments in which the IRM compound may be administered after actual or suspected exposure to the infectious agent, administration of the IRM can begin prior to the detectable onset of symptoms resulting from infection by the infectious agent. In such embodiments, the course of treatment may conclude before the onset of any symptoms. Alternatively, in some embodiments, the course of treatment may begin prior to the onset of symptoms and continue even after the subject experiences symptoms resulting from

infection by the infectious agent. In such cases, it is expected that the extent of infection will be significantly reduced by practicing the methods of the invention, thereby limiting the severity, extent, and/or duration of symptoms.

In other alternative embodiments, the IRM compound may be administered before an anticipated exposure to an infectious agent, and again after the exposure to the infectious agent has, or is suspected to have, occurred. Again, in such embodiments, the course of treatment may conclude prior to, or may continue after, the onset of any symptoms resulting from infection by the infectious agent.

The methods of the present invention may be performed on any suitable subject. Suitable subjects include but are not limited to animals such as but not limited to humans, non-human primates, rodents, dogs, cats, horses, pigs, sheep, goats, or cows.

Examples

The following examples have been selected merely to further illustrate features, advantages, and other details of the invention. It is to be expressly understood, however, that while the examples serve this purpose, the particular materials and amounts used as well as other conditions and details are not to be construed in a matter that would unduly limit the scope of this invention. Unless otherwise provided, all percentages are given as w/w%.

The IRM compound used in the examples is N-{2-[4-amino-2-(ethoxymethyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl]-1,1-dimethylethyl}methanesulfonamide, the synthesis of which is described in example 268 of U.S. Patent No. 6,677,349.

Example 1

IRM compound was prepared as a 0.375% solution formulation capable of being nasally administered via a spray pump. The formulation vehicle was prepared as follows:

Table 1

Excipient	w/w%
Carboxymethyl cellulose sodium, low viscosity, USP (Spectrum Chemicals and Laboratory Products, Inc., Gardena, CA,)	0.1
Benzalkonium chloride, Ph. Eur. (Fluka, Buchs Switzerland)	0.02
Disodium EDTA, USP (Spectrum Chemicals)	0.1
L-Lactic acid, Purac (Lincolnshire, IL)	1.53
PEG 400, NF (Spectrum Chemicals)	15
1 N NaOH, NF (Spectrum Chemicals)	qs
Water	qs
Total	100.00
pH	4.0

Carboxymethyl cellulose sodium, low viscosity, (CMC) was hydrated in water (about 50% of total) for 20 minutes with stirring. The EDTA was added and dissolved. The CMC/EDTA solution was mixed with the benzalkonium chloride to form a CMC/EDTA/BAC solution. Separately, the lactic acid and PEG 400 were mixed with water. For the IRM formulation, IRM compound was dissolved into the lactic acid/PEG 400 solution. The CMC/EDTA/BAC solution was mixed with lactic acid/PEG 400 solution to prepare the Vehicle formulation. The CMC/EDTA/BAC solution was mixed with lactic acid/PEG 400/IRM solution to prepare the IRM formulation. 1 N NaOH was added, as necessary, to adjust each formulation to a pH of 4.0. Finally, water was added to each formulation to adjust to the final formulation weight.

Example 2

Fisher 344 rats (Charles River Laboratories, Raleigh, NC) were divided into six treatment groups. Rats in each group were infected intranasally with humanized, non-lethal influenza virus. 24 hours after infection, viral titers were measured in nasal lavage fluid and whole lung homogenates. The influenza virus and methods for measuring viral titers are described in Burleson, Gary L., "Influenza Virus Host Resistance Model for Assessment of Immunotoxicity, Immunostimulation, and Antiviral Compounds," *Methods in Immunology 2*:181-202, Wiley-Liss Inc., 1995..

Each of the six treatment groups received a different pre-infection treatment. Rats in each group received the treatment indicated in Table 2. The results are summarized in Figure 1 and Figure 2.

Table 2

Group	Treatment
1	Vehicle formulation (Table 1), 50 μL (25 μL per nare), 1x*
2	Interferon-α (rat recombinant IFN-α, Cat. No. PRP13, Serotec Inc., Raleigh, NC), 10,000 IU, 1x
3	IRM formulation (Table 1), 50 μL (25 μL per nare), 1x
4	Vehicle formulation (Table 1), 50 μL (25 μL per nare), 2x**
5	Interferon-α , 10,000 IU, 2x (Day –1: Product No. RR2030U, Pierce Biotechnology, Inc., Rockford, IL; Day 0: Serotec Inc. Cat. No. PRP13)
6	IRM formulation (Table 1), 50 μL (25 μL per nare), 2x

*1x: one dose of treatment provided four hours before viral infection.

**2x: one dose of treatment 24 hours (Day –1) before viral infection, second treatment four hours before viral infection (Day 0).

Example 3

Thirty-five mL of blood from volunteer donors was layered over 15 mL of room temperature Histopaque-1077 Hybri-Max (Sigma Chemical Co., St. Lois, MO) in an Accuspin tube (Sigma Chemical Co.) and centrifuged at room temperature, 800 x g for 15 minutes (Beckman centrifuge). PBMC band (2nd layer) was removed and washed twice with Hanks Balanced Salt Solution (HBSS). Final cell pellet was resuspended in 10% Fetal Bovine Serum and 1X Penicillin/Streptomycin in RPMI-1640. Suspension density was determined using trypan blue and a hemacytometer. Density was brought to 1 X 10^6 cells/milliliter in growth media, 10 mLs was placed in a T25 tissue culture flask, and suspension was allowed to equilibrate at 37°C in a humidified incubator with 5-7% CO_2 for 1 hour.

IRM compound was added in growth media to desired final concentration. Supernatants were harvested after 24 hours and frozen until tested. Five concentrations of IRM compound in test media or as PBMC supernatant solutions (sup) were prepared for testing.

Viruses:

Human coronavirus (HcoV), strain OC43: Obtained from the American Type Culture Collection (ATCC), Manassas, Virginia. Pools of virus made up in BSC-1 cells.

Influenza A/Panama/2007/99 (H3N2): Obtained from the Centers for Disease Control and Prevention (CDC), Atlanta, GA. Virus stocks were prepared in MDCK cells.

Influenza A/New Caledonia/20/99 (H1N1): Obtained from the CDC, Atlanta, GA. Virus stocks were prepared in MDCK cells.

Severe acute respiratory syndrome (SARS) coronavirus, strain Urbani. Obtained from the CDC, Atlanta, GA. A pool was prepared in African green monkey kidney (Vero 76) cells.

Cells and Media:

BSC-1 cells (used for human corona viruses). Growth medium is MEM with 5% fetal bovine serum (FBS) and 0.1% $NaHCO_3$. Test medium is MEM with 2% FBS, 0.1% $NaHCO_3$ and 50 μg/mL gentamycin.

MDCK cells (Maden Darby canine kidney, used for influenza virus), obtained from the ATCC. Growth medium is MEM with 5% FBS and 0.1% $NaHCO_3$. Test medium is MEM without serum, 0.18% $NaHCO_3$, 20 μg trypsin/mL, 2.0 μg EDTA/mL, and 50 μg gentamycin/mL.

Vero 76 cells (used for SARS coronavirus), obtained from the ATCC. Growth medium is MEM with 5% fetal bovine serum (FBS) and 0.1% $NaHCO_3$. Test medium is MEM with 2% FBS, 0.1% $NaHCO_3$ and 50 μg gentamycin/mL.

Antiviral Testing Procedure:

For all viruses, cells were seeded to 96-well flat-bottomed tissue culture plates (Corning Glass Works, Corning, NY), 0.2 mL/well, at the proper cell concentration, and incubated overnight at 37° C in order to establish a cell monolayer. When the monolayer was established, the growth medium was decanted. Each dilution of the compound was added in a volume of 0.1 mL to a total of 5 wells/dilution; approximately 5 min later 0.1 mL of virus in test medium was added to 3 wells per dilution, and sterile virus diluent added to the remaining wells. Six wells were exposed to drug diluent and virus to serve as virus controls, and 6 wells were exposed to drug diluent and sterile virus diluent to serve

as normal cell controls. The plates were sealed with plastic wrap and incubated in a humidified incubator with 5% CO_2, 95% air atmosphere at 37°C for the time required for viral cytopathic effect (CPE) to fully develop in the virus control wells.

Cells were then examined microscopically for CPE, this being scored from 0 (normal cells) to 4 (maximal, 100%, CPE). The cells in the toxicity control wells were observed microscopically for morphologic changes attributed to cytotoxicity. This cytotoxicity was also graded at T (100% toxicity, complete cell sloughing from plate), PVH (60% cytotoxicity), P(40% cytotoxicity), PS (20% cytotoxicity), and 0 (normal cells). The 50% effective dose (EC_{50}) and 50% cytotoxic dose (CC_{50}) were calculated by regression analysis of the virus CPE data and the toxicity control data, respectively. The selective index (SI) for each compound tested is calculated by the formula: SI =CC_{50} ÷EC_{50}.

After the plates were read visually for cytopathology and toxicity, the cells were then stained with neutral red to verify the visual determinations. To do this, 0.1 mL of sterile neutral red (0.034% in physiological saline solution) was added to each well. The plates were wrapped in aluminum foil to eliminate light exposure and placed at 37° C for one to two hours. All medium was removed and the cells gently washed 2x (0.2 mL/well for each wash) with phosphate buffered saline. The plates were inverted and allowed to drain on a paper towel. Neutral red was extracted from the cells by adding 0.2 mL of an equal volume mixture of absolute ethanol and Sorensen's citrate buffer, pH4, to each well and placing the plates in the dark at room temperature for 30 minutes. The contents of each well were mixed gently and the O.D. values of each well were obtained by reading the plates at 540 nm with a Model EL309 microplate reader (Bio-Tek Instruments, Inc., Winooski, VT). The EC_{50} and CC_{50} are calculated by regression analysis. The SI for each compound tested was again calculated by the formula: SI=CC_{50} ÷EC_{50}.

A known active substance was run in the same manner as above for each batch of compounds tested. For influenza viruses, ribavirin was used. For human coronavirus (HCoV) and SARS, ALFERON (Hemispherx Biopharma, Inc., Philadelphia, PA) was used. In these studies, selected cell types were pretreated with supernatant solutions from IRM-stimulated PBMC cultures or with media containing solubilized drug and then exposed to different types of respiratory viruses. Viral replication was then determined by viral cytopathic effect (CPE) analysis and neutral red (NR) analysis for confirmation.

Cytotoxic concentrations were also determined and were described by the concentration that produced 50% cell death. Antiviral activity was only considered to exist when the concentration for 50% cytotoxicity was at least 3 times greater than the effective concentration for 50% inhibition in the CPE assay (i.e, SI > 3). Results are summarized in Table 3.

Table 3

Virus	Treatment	CC_{50} (μM)	EC_{50} (μM)	SI (CC_{50}/EC_{50})
Influenza A (H3N2)	PBMC supernatant	>1.5	<0.0015	>1000
	IRM	>300	>300	0
	Ribavirin	>450	24	>19
Influenza A (H1N1)	PBMC supernatant	>1.5	<0.0015	>1000
	IRM	>300	>300	0
	Ribavirin	>450	14	>32
SARS	PBMC supernatant	>1.5	0.15	>10
	IRM	>150	>3	50
	ALFERON	>32,000	<32	>1000
HCoV	PBMC supernatant	>1.5	0.17	>9
	IRM	>150	>150	0
	ALFERON	>32,000	32	>1000

PBMC supernatant solutions from cells stimulated with IRM compound had significant activity against SARS, both of the influenza viruses tested, and human coronavirus. IRM compound, alone, possessed significant direct activity against SARS.

The complete disclosures of the patents, patent documents and publications cited herein are incorporated by reference in their entirety as if each were individually incorporated. In case of conflict, the present specification, including definitions, shall control.

Various modifications and alterations to this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention. Illustrative embodiments and examples are provided as examples only and are not intended to limit the scope of the present invention. The scope of the invention is limited only by the claims set forth as follows.

What is Claimed is:

1. A method of providing prophylaxis to a subject against an infectious agent comprising topically administering to the respiratory tract of a subject an IRM compound in an amount effective to reduce infection by the agent.

2. The method of claim 1 wherein the IRM compound is administered nasally.

3. The method of claim 1 wherein the IRM compound is administered orally.

4. The method of claim 1 wherein the IRM compound is administered from about 72 hours prior to exposure to the infectious agent to about 72 hours after exposure to the infectious agent.

5. The method of claim 4 wherein the IRM compound is administered prior to and after exposure to the infectious agent.

6. The method of claim 4 wherein the IRM compound is administered prior to but not after exposure to the infectious agent.

7. The method of claim 4 wherein the IRM compound is administered after but not prior to exposure to the infectious agent.

8. The method of claim 4 wherein the IRM compound is administered in from 1 to about 12 doses.

9. The method of claim 8 wherein the IRM compound is administered in from 1 to about 3 doses.

10. The method of claim 9 wherein the IRM compound is administered in 1 dose.

11. The method of claim 10 wherein the infectious agent comprises a virus, a bacterium, a parasite, or a prion.

12. The method of claim 11 wherein the virus is an influenza virus.

13. The method of claim 11 wherein the virus is a coronavirus.

14. The method of claim 13 wherein the virus is a SARS coronavirus.

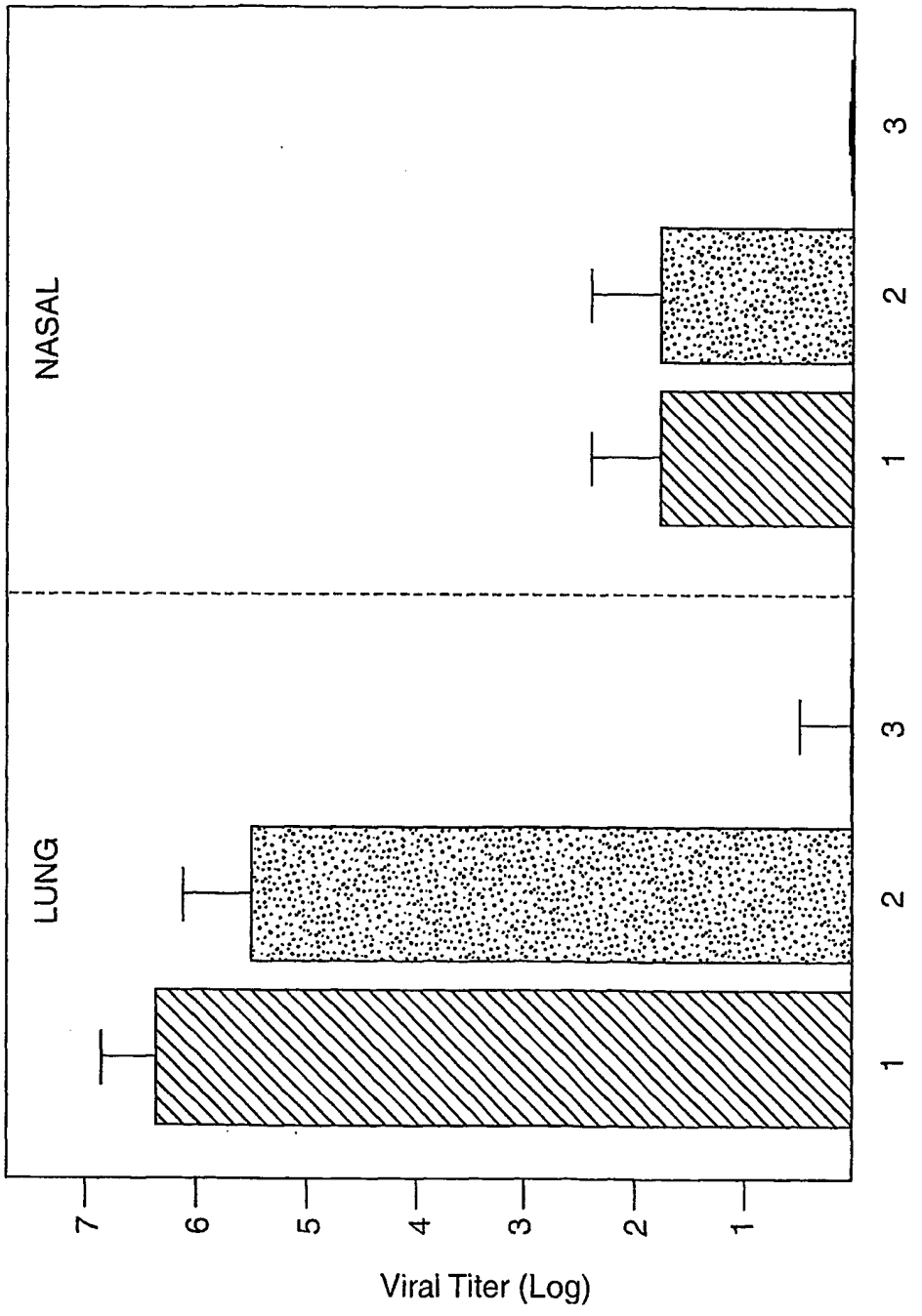

Fig. 1

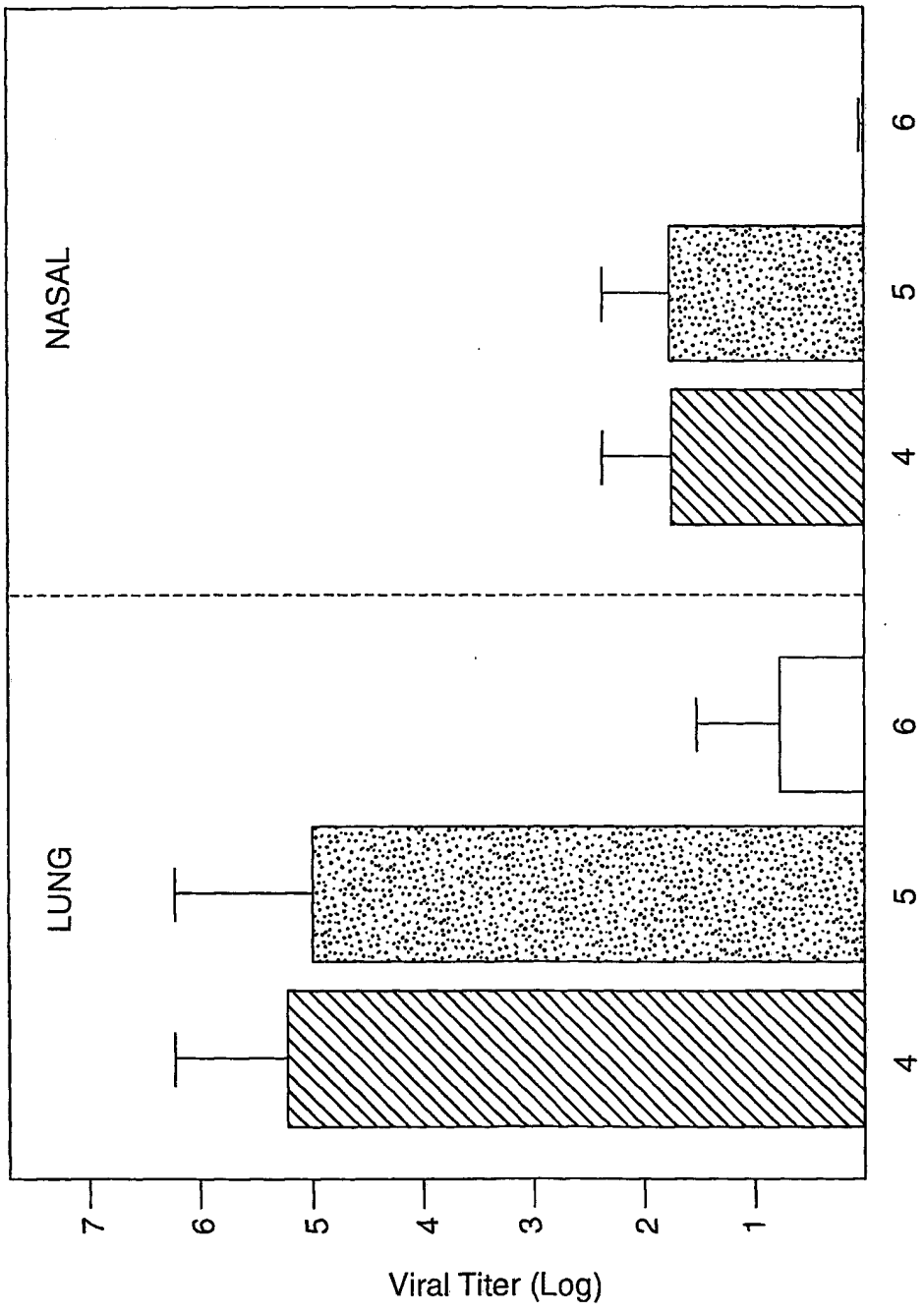

Fig. 2